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PRINCIPAL INVESTIGATOR: Ren Xu, Ph.D.
Mina J. Bissell

CONTRACTING ORGANIZATION: Lawrence Berkeley National Laboratory
Berkeley, CA 94720

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6. AUTHOR(S)
Ren Xu, Ph.D.
Mina J. Bissell

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Lawrence Berkeley National Laboratory
Berkeley, CA 94720

E-Mail: RXu@lbl.gov

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Relationship between chromatin remodeling and mammary tissue-specific gene transcription is not well understood. Using milk protein β-casein as a marker, we investigate how extracellular matrix (ECM) and lactogenic hormone control transcription factors activity, and elucidate the role of histone acetylation and ATP-dependent chromatin remodeling in the transcriptional regulation. By ChIP assays, we show that ECM cooperates with prolactin to induce binding of Stat5 and C/EBPβ in the β-casein promoter. We also show that the levels of acetylated histones increase in the β-casein promoter. However, increasing acetylated histone levels in the promoter region by TSA treatment failed to induce β-casein expression, suggesting histone acetylation is not sufficient for the gene transcription. Introduction of the ATPase-deficient SWI/SNF complex significantly blocked β-casein expression, indicating that ATP-dependent chromatin remodeling is required for the transcriptional activation of this gene. Taken together, these observations indicate that β-casein expression requires the concerted action of both transcription and chromatin remodeling factors.

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Introduction:

In addition to transcription factors, modulation of chromatin structure by histone modifications and ATP-dependent remodeling has been implicated in transcriptional regulation of tissue specific genes (6). Histone modifying enzymes and ATP-dependent SWI/SNF-like complexes are believed to be recruited to the promoter region of genes through their association with transcription factors. Once recruited, these cofactors facilitate the activation and/or repression of specific gene transcription (4, 16). β-casein, a functional differentiation marker of mammary epithelial cells (MECs) (12), has been widely used to study the transcriptional regulation of mammary tissue-specific genes. In primary mouse mammary epithelial cells, as well as immortalized Eph4 and CID-9 cell lines, transcription of β-casein was shown to depend on the signals generated from both lactogenic hormones and laminin-enriched ECM (ECM) (8, 11, 13). Experiments in vivo and in culture have demonstrated that the murine β-casein promoter contains binding sites for Stat5, C/EBPβ, and GR, and that the recruitment of these factors to the promoter is critical for β-casein transcription (3, 11, 15, 17). In last year annual report, we showed that ECM and prolactin induced Stat5 phosphorylation and nuclear translocation in Eph4 cells. Chromatin immunoprecipitation (ChIP) assay demonstrated that both Stat5 and C/EBPβ bound to the β-casein promoter after the induction. However, it is still unclear whether chromatin remodeling is involved in β-casein transcription.

By ChIP assay, we showed that the levels of acetylated histones increased in the β-casein promoter. However, increasing acetylated histone levels in the promoter region by TSA treatment failed to induce β-casein expression, suggesting histone acetylation is not sufficient for the gene transcription. Introduction of the ATPase-deficient SWI/SNF complex significantly blocked β-casein expression, indicating that ATP-dependent chromatin remodeling is required for the transcriptional activation of this gene. Taken together, these observations indicate that β-casein expression requires the concerted action of both transcription and chromatin remodeling factors.

Body

Part I: Stat5, C/EBPβ, Brg1 and RNA polymerase II is recruited to the β-casein promoter after ECM and prolactin treatment.

Using ChIP assay, we examined the protein association with the chromatin in Eph4 cells before and after ECM and prolactin treatment. The results showed that the levels of Stat5 and C/EBPβ in the β-casein promoter were increased 2-3 fold after induction, while the interaction between the promoter and GR remained the same regardless of treatment (Fig 1A). In addition, control β-amylase promoter DNA sequences were not detected in all samples (Fig 1B). Thus, exposure of mammary epithelial cells to ECM and prolactin increases nuclear Stat5 levels and induces its binding to the β-casein promoter.

Rent studies have shown that ATP-dependent chromatin remodeling is important for some gene transcription in eukaryotes (7). No evidence has been presented showing an involvement of ATP-dependent chromatin remodeling factors in β-casein expression. Therefore, we investigated whether SWI/SNF remodeling complexes are associated with β-casein promoter by performing ChIP assays using an antibody against Brg1. Results showed that Brg1 binding activity in the promoter region was moderately enhanced after ECM and prolactin treatment, while no binding of Brg1 could be detected in β-amylase promoter (Figure 1B, C). PCR analyses of the ChIP DNA also showed an increased association of RNA polymerase II with the β-casein promoter in response to the treatment (Fig 1B). These results suggest that the recruitment of transcription factors and SWI/SNF complexes to the promoter may induce the association of RNA polymerase II and thus initiate transcription.

Part II: Histone acetylation is involved in but not sufficient to β-casein transcription.

We showed previously that treatment with histone deacetylase inhibitors activates stably integrated BCE-1 in a mammary epithelial cell line (CID-9) in the absence of ECM, suggesting that histone acetylation plays a role in transcriptional regulation of the bovine casein enhancer (9). Surprisingly, however, the same treatment was shown later to inhibit transcriptional activation of the endogenous β-casein gene (10). We therefore had to determine if histone acetylation is directly involved in endogenous β-casein transcriptional regulation. A ChIP assay was performed with antibodies against acetylated H3 and H4. The binding of both acetylated H3 and H4 to the β-casein promoter, but not to the β-amylase promoter, was enhanced upon β-casein transcriptional activation (Figure 2A). We next determined whether the increase of acetylated histone level in the promoter was sufficient to induce β-casein transcription. After Eph4 cells were treated with TSA in GIH medium for 2 days, RT-PCR and ChIP analysis were conducted. Results revealed that β-casein transcription was not induced after the treatment (Figure 2B). However, TSA treatment caused global histone acetylation in the chromatin, as well as along the β-casein promoter region (Figure 2C), suggesting that

histone acetylation contributes to, but is not sufficient for the induction of β-casein transcription.

Part III: The recruitment of transcription factors and histone acetylation in the β-casein promoter depends on both ECM and prolactin signals.

We have shown that transcription factors and SWI/SNF complexes were recruited to the β-casein promoter, and that acetylated histone levels increased in the promoter region upon ECM and prolactin treatment. To determine whether ECM and prolactin control these events separately or cooperatively, we performed ChIP analysis after the cells were treated with ECM and/or prolactin. Results showed that Stat5 bound to the promoter in the ECM and prolactin treated cells, but treatment with either component failed to induce the recruitment. It is consistent with the western data that Stat5 nuclear translocation depends on both signals. Combined ECM and prolactin treatment was necessary to induce binding of C/EBPβ to the promoter. Whereas ECM treatment alone slightly enhanced C/EBPβ binding to the promoter, the enhancement was not consistently observed from assay to assay. Recruitment of Brg1 and RNA polymerase II, and induction of histone acetylation along the β-casein promoter also required both ECM and prolactin (Figure 3). These results established that ECM cooperates with prolactin to induce histone acetylation, as well as the binding of transcription factors and the SWI/SNF complex to the β-casein promoter.

Part IV: ATPase activity of SWI/SNF complex is critical for β-casein expression.

In addition to histone modifications, ATP-dependent chromatin remodeling SWI/SNF complexes have been shown to play an important role in transcriptional regulation of eukaryotic gene (1, 7). Mammalian cells contain at least two SWI/SNF-like complexes that share many of the same subunits. However, these complexes are distinguished from one another by their ATPase subunits, Brg1 and Brm1 (5). Studies with ATPase-deficient Brg1 have demonstrated that the ATPase activity of SWI/SNF complexes is required for the expression of tissue-specific genes in muscle and adipose tissue (1, 2, 14). To examine the role of SWI/SNF during β-casein transcriptional activation, we generated an Eph4 cell line that conditionally expressed Flag-tagged DN-Brg1 under control of the tetracycline-repressible transactivator. This allowed us to induce the DN-Brg1 expression by withdrawal of tetracycline from culture medium (2). Western results indicate that the level of DNBrg1 expression in the transfected Eph4 cells (Clone 12) was drastically increased 2 days after withdrawal of tetracycline (Figure 4A). RT-PCR and quantitative PCR analysis showed that β-casein gene transcription was not affected in vector control cells by plus or minus tetracycline (Figure 4B, C). However, β-casein mRNA levels were decreased 5 fold in DN-Brg1-expressing cells by withdrawal tetracycline (Figure 4B, C).

Future Work:

We have shown that both histone acetylation and ATP dependent chromatin remodeling are involved in β-casein transcriptional regulation. We will further study whether other milk protein genes are also regulated by the same mechanism. We have defined a pathway from extracellular cues to chromatin remodeling that controls β-casein transcription. Now, we are writing a manuscript based these results.

Key research accomplishments:

1, Both transcription factors and chromatin remodeling complexes were recruited to the β-casein promoter upon ECM and prolactin treatment.

2, Although acetylated histone levels increased in the promoter after induction, enhancing the acetylation by HDAC inhibitor failed to induce β-casein expression. These results suggest histone acetylation is not sufficient for β-casein transcriptional activation.

3, ECM cooperates with prolactin to induce histone acetylation, as well as the binding of transcription factors and SWI/SNF complexes in the β-casein promoter.

4, The transcription of β-casein gene depends on the ATPase activity of SWI/SNF complex.

Reportable Outcomes:

One abstract was present in AACR Chromatin, Chromosomes, and Cancer Epigenetics meeting.

One manuscript is in preparation

Conclusion:

In this report, we showed that ECM cooperates with prolactin to induce histone acetylation, as well as the binding of transcription factors and SWI/SNF complexes in the β-casein promoter. Histone acetylation in the promoter region alone is not sufficient to induce β-casein transcription. Deficient the ATPase activity of SWI/SNF complex significant inhibits β-casein expression. Take together; these results indicate that histone acetylation, SWI/SNF chromatin remodeling complex, and transcription factors cooperatively regulate β-casein transcription.

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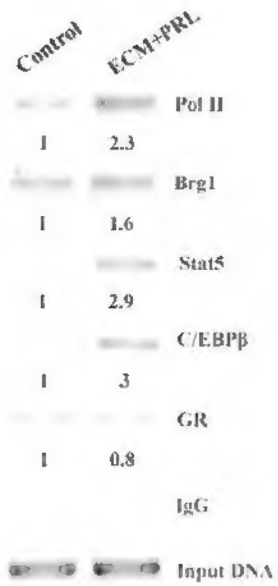
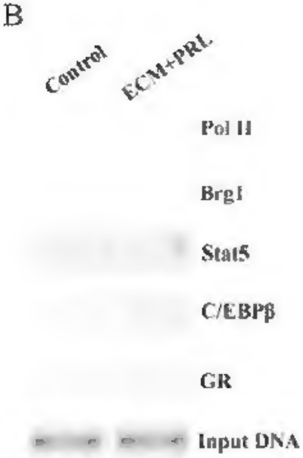
Figures 1:

A

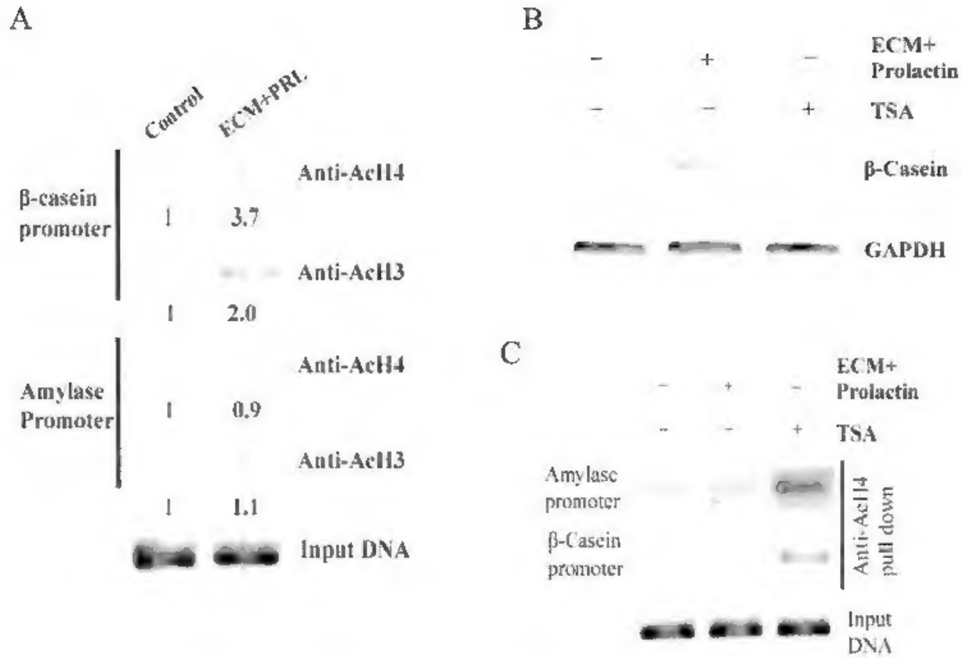


B



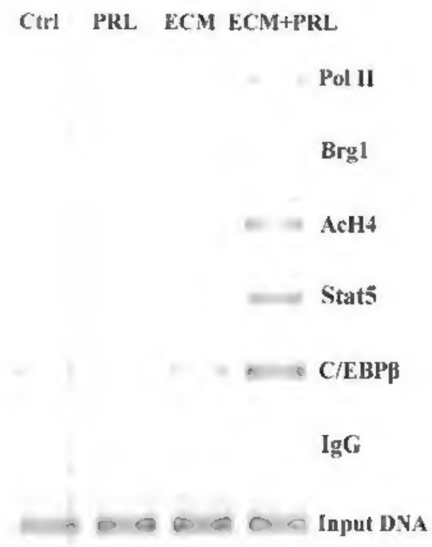
(A) Chromatin was immunoprecipitated with antibodies against Stat5, C/EBPβ, GR, Brg1, and RNA Polymerase II, and the level of β-casein promoter sequence in the ChIP DNA was measured by PCR analysis. Chromatin DNA (10 μl) was aliquoted before immunoprecipitation as an input control. Ratios of precipitated DNA over input DNA were calculated as a relative fold shown below each sample. ChIP DNA immunoprecipitated with normal rabbit IgG was used as a negative control. (B) PCR analysis of a region in the β-Amylase promoter was performed on the same ChIP DNA samples as a negative control. The data were representative of three experiments.

Figure 2:



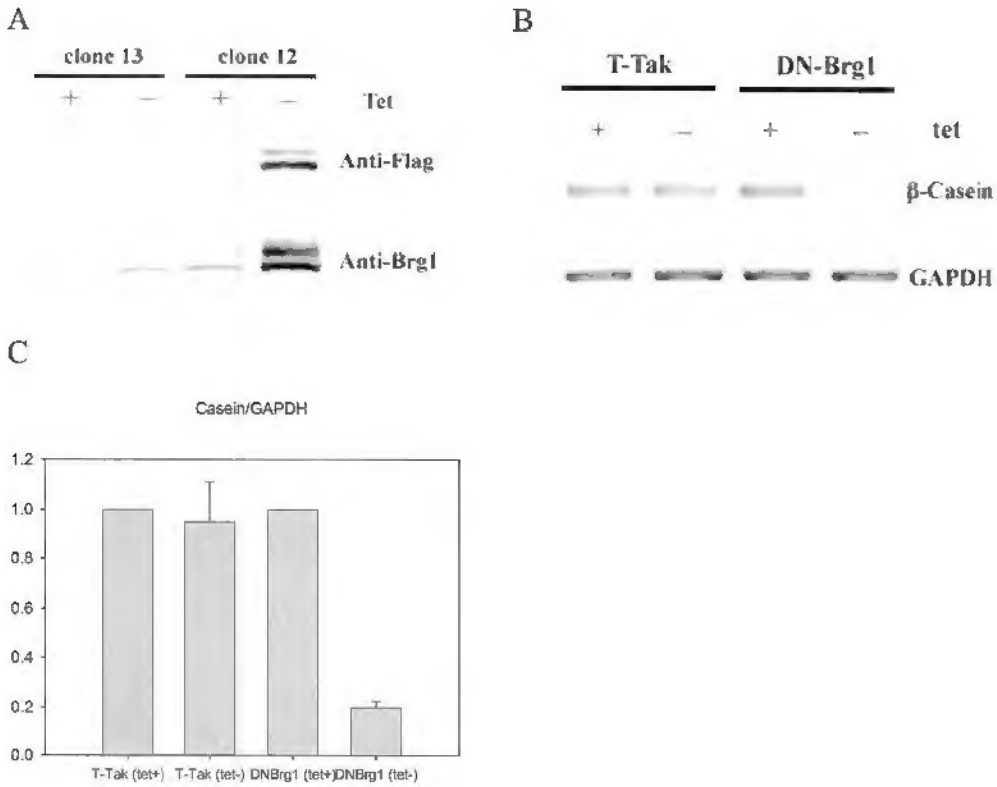
Histone acetylation is involved in β-casein transcription. (A) ChIP was performed with antibodies against acetylated histone H4 and H3. The level of β-casein promoter sequence was measured by PCR, and β-Amylase promoter was used as negative control. (B) β-casein mRNA levels were measured by RT-PCR after TSA treatment. (C) AcH4 and AcH3 levels in the promoter region were detected by the ChIP assay after Eph4 cells were treated with TSA (80 nM) for 2 days. These experiments were performed a minimum of two times.

Figure 3:



The recruitment of transcription factors and histone acetylation to the β-casein promoter depends on both ECM and prolactin. After Eph4 cells were treated with prolactin, ECM, or prolactin plus ECM, chromatin was immunoprecipitated with specific antibodies. The level of β-casein promoter sequence was measured by PCR analysis. ChIP DNA immunoprecipitated with normal rabbit IgG was used as negative control. These results were representative of two separate experiments.

Figure 4:



DN-Brg1 expression in Eph4 cells inhibits transcription of the β-casein gene. (A) After transfected cells were cultured in media with or without tetracycline for 2 days, DN-Brg1 expression was checked by western blot analysis using antibodies against Flag and Brg1. Clones 12 and 13 represents the positive and negative clones, respectively. (B, C) After the cells were cultured with or without tetracycline for two days, β-casein transcription was induced by ECM and prolactin for another two days. T-Tak is a vector control clone. DN-Brg1 is a DN-Brg1 expressing clone. β-casein mRNA levels were detected by RT-PCR (B) and real-time PCR (C), and normalized to GAPDH levels.